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Structural Motif of the DNA Binding Domain of γδ-Resolvase Characterized by Affinity Cleaving*

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The DNA binding domain of γδ-resolvase, residues 141–183, is thought to bind DNA by a helix-turn-helix motif based on sequence similarities with other known DNA binding proteins. Incorporation of the DNA cleaving moiety, EDTA·Fe, at the NH_2 and COOH termini of γδ(141–183) allows the positions of these residues relative to the DNA bases at three resolvase binding sites, each consisting of inverted copies of an imperfectly conserved 9-base pair sequence, to be mapped by high resolution gel electrophoresis. The cleavage data for EDTA-γδ(141–183) reveals that the NH_2 terminus of the DNA binding domain of γδ-resolvase is bound proximal to the minor groove of DNA near the center of the resolvase binding sites. Cleavage by EDTA·Fe attached to a lysine side chain (Asn[183]→Lys[183]) at the COOH terminus of γδ(141–183) reveals that the putative recognition helix is in the adjacent major groove on the same face of the helix, oriented toward the center of the inverted repeats.

The structural class of DNA binding proteins best characterized by crystallographic studies contains the helix-turn-helix motif. Comparison of the three-dimensonal structures of λ-cro, λ-repressor, and catabolite gene activator protein led to the postulate that a conserved α-helix-turn-α-helix motif is involved in recognition of DNA in the major groove and may be a common structural motif for sequence-specific DNA affinity (Anderson *et al.*, 1981; McKay and Steitz, 1981; Pabo and Lewis, 1982; McKay *et al.*, 1982; Ohlendorf and Matthews, 1983; Pabo and Sauer, 1984; Schevitz *et al.*, 1985). The x-ray structure determination of three proteins containing helix-turn-helix motifs bound to their DNA operator sites elucidates the DNA binding domain of the 434 repressor (1–69) (Anderson *et al.*, 1985, 1987; Aggarwal *et al.*, 1988), the DNA binding domain of λ-repressor (1–92) (Jordan and Pabo, 1988), and the trp repressor (Otwinowski *et al.*, 1988). These high resolution crystallographic views of repressor-operator complexes reveal the complexity of protein-DNA interactions. The protein-DNA interface includes protein contacts to the sugar-phosphate backbone as well as to base pairs in the major groove. A particular side chain can contact several base pairs, and several side chains can cooperate to recognize a single base. Moreover, sequence-dependent ability of DNA to adopt the required conformation appears important for site-specific recognition. The combination of direct protein-DNA contacts mediated by multiple hydrogen bonds and the sequence-dependent conformational effects in DNA limits our ability to make detailed structural predictions. In the absence of high resolution crystallographic and nuclear magnetic resonance data, affinity cleavage methods can be utilized to correlate sequence similarities with known structural classes (Sluka *et al.*, 1987, 1990; Mack *et al.*, 1990; Oakley and Devan, 1990).

The γδ-resolvase is a 183-residue protein encoded by the transposable element, γδ, a member of the Tn3 family of bacterial transposons (Grindley and Reed, 1985; Heffron, 1983). Resolvase is both a site-specific recombinational protein and a repressor. It interacts with a 120-bp[1] site named *res* which lies within the region between the divergently transcribed tnpA and tnpR genes of the γδ transposon. Resolvase protects three binding sites within *res* from nuclease digestion (Grindley *et al.*, 1982; Kitts *et al.*, 1983). Each binding site consists of inverted repeats of a 9-base pair segment consensus sequence, TGTCYNNTA (where Y is a pyrimidine and N means any base), separated by a variable spacer of 7, 10, or 16 bp (Grindley *et al.*, 1982). Site I, which has a 10-bp spacer, contains the recombination of cross-over point; all three sites are required for efficient recombination (Grindley *et al.*, 1982; Kitts *et al.*, 1983; Wells and Grindley, 1984). γδ-Resolvase has been shown to induce a structual change in the DNA at site I that corresponds to a bend (Hatfull *et al.*, 1987; Salvo and Grindley, 1988). The interactions between γδ-resolvase and DNA have been examined by methylation and ethylation interference studies (Falvey and Grindley, 1987). Major groove methylations within the 9-bp recognition sequence as well as ethylation of phosphates within and adjacent to this region were found to inhibit resolvase binding. Furthermore, inhibition of resolution by methylation of adenine at the center of site I suggests that minor groove contacts near the cross-over may be required for resolution activity.

γδ-Resolvase can be cleaved by chymotrypsin into two fragments (Abdel-Meguid *et al.*, 1984). The NH_2-terminal fragment, γδ(1–140), which is thought to contain the protein-protein contacts and be responsible for the enzymatic activity, does not bind DNA. The 43-residue COOH-terminal fragment γδ(141–183) binds specifically but independently to both halves of all three DNA sites to which resolvase binds. However, unlike native resolvase, which binds to all three complete

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[1] The abbreviations used are: bp, base pair(s); DTT, dithiothreitol; *t*-Boc, *t*-butoxycarbonyl; DMF, dimethylformamide; DIEA, diisopropylethylamine; HOBt, *N*-hydroxybenzotriazole; DCM, dichloromethane; HPLC, high performance liquid chromatography; Fmoc, 9-fluorenylmethyloxycarbonyl; DNP, dinitrophenol; MPE, methidiumpropyl-EDTA; OBzl, benzyl ester; Bzl, benzyl; Cl-Z, 2-chlorobenzyloxycarbonyl; Tos, 4-toluenesulfonyl.

sites with equal affinities, γδ(141–183) binds to each of the six half-sites with different affinities (Abdel-Meguid *et al.*, 1984). Ethylation interference experiments reveal that phosphate contacts made by the COOH-terminal DNA binding domain are similar to those of the intact resolvase with the exception of a single phosphate at the inside of each contact region (Rimphanitchayakit *et al.*, 1989). Phosphate contact extends across adjacent major and minor grooves on one face of the DNA helix. The minimal binding segment is a 12-bp sequence that includes the 9-bp inverted repeat. γδ(141–183) contains a high degree of sequence similarlity with the helix-turn-helix regions of several DNA binding proteins (Pabo and Sauer, 1984). Based on sequence similarities with other DNA

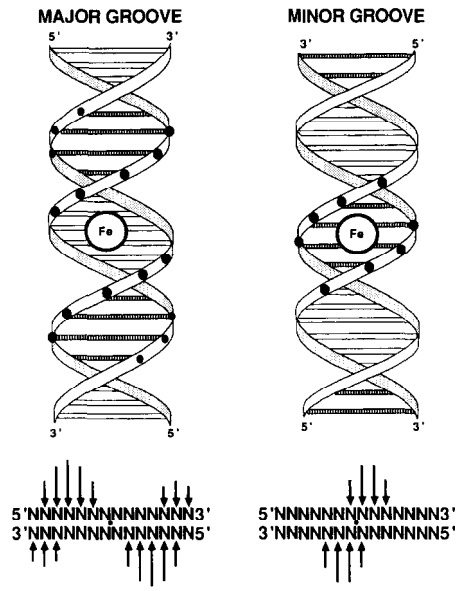

FIG. 1. **Cleavage patterns produced by a diffusible oxidant generated by EDTA·Fe located in the major (Moser and Dervan, 1987; Griffin and Dervan, 1989) and minor (Taylor *et al.*, 1984; Dervan, 1986) grooves of right-handed DNA.** *Filled circles* represent points of cleavage along the phosphodiester backbone. Sizes of circles represent extent of cleavage.

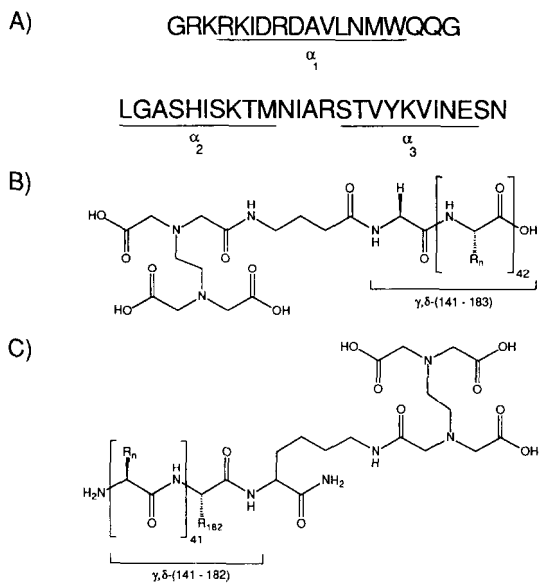

FIG. 2. *A*, the 43-amino-acid DNA binding domain of γδ-resolvase. *Underlined regions* are possible α-helices assigned according to a secondary structure predicting algorithm (Garnier *et al.*, 1978) (*B*) EDTA-γδ(141–183), (*C*) γδ(141–183, Asn^{183}→Lys^{183})-EDTA.

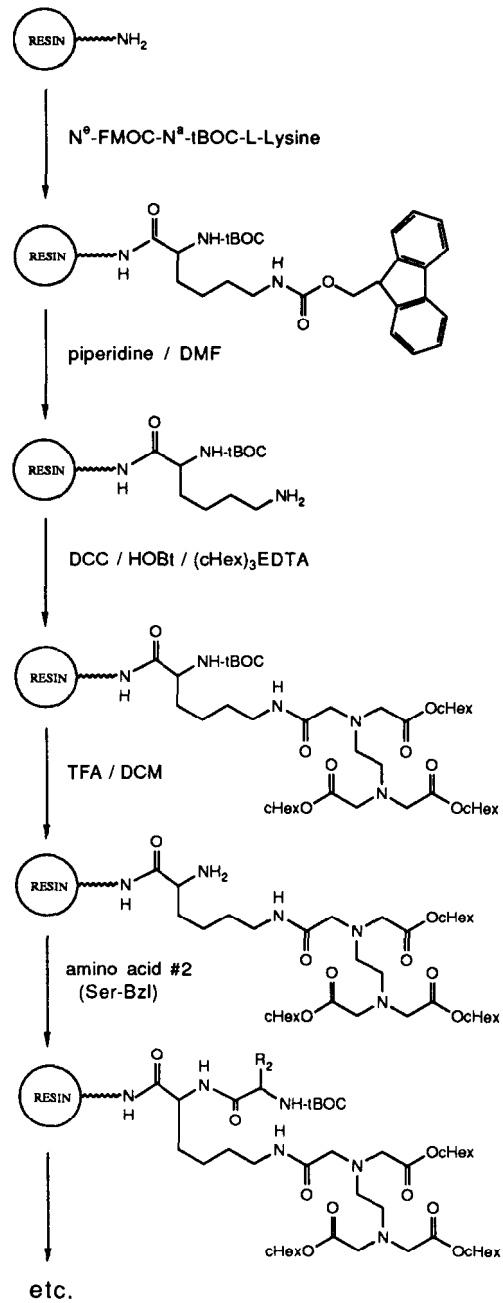

FIG. 3. **Synthetic scheme for the attachment of the tricyclohexyl (*cHex*) ester of EDTA to the ε-amino group of Lys^{183} on *p*-methylbenzhydrylamine resin (Sluka *et al.*, 1990b).** *DCC*, dicyclohexylcarbodiimide; *TFA*, trifluoroacetic acid; *OBzl*, benzyloxy.

binding proteins, it has been proposed that resolvase uses the helix-turn-helix motif in the major groove for sequence-specific DNA binding with its NH_2 terminus oriented toward the center of each binding site (Abdel-Meguid *et al.*, 1984; Rimphanitchayakit *et al.*, 1989).

Incorporation of the DNA cleaving moiety, EDTA·Fe, at discrete amino acid residues along a protein allows the positions of those residues in the protein-DNA complex relative to the DNA bases to be mapped to nucleotide resolution (Sluka *et al.*, 1987). Following chemical activation with a reducing agent such as dithiothreitol (DTT), EDTA·Fe localized at a specific DNA binding site cleaves both DNA strands, typically covering 4–6 base pairs via a diffusible species (Schultz *et al.*, 1982; Taylor *et al.*, 1984; Dervan, 1986). Due to the right-handed nature of double-helical DNA, the

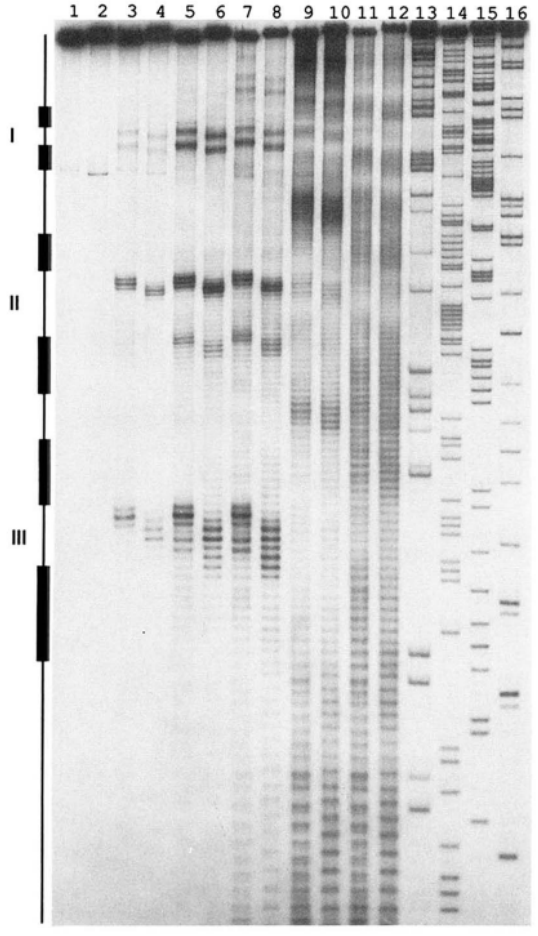

FIG. 4. **Autoradiogram of a high resolution denaturing polyacrylamide gel containing ^{32}P-end-labeled fragments from pRW80.** *Bars* on the *left* indicate the position of the three binding sites I, II, and III (each consisting of imperfectly conserved inverted repeats) for γδ-resolvase with *res*. Odd- and even-numbered *lanes 1–12* contain 5′- and 3′-labeled DNA, respectively. *Lanes 1* and *2*, intact DNA; *lanes 3* and *4*, EDTA-γδ(141–183) (0.5 μM); *lanes 5* and *6*, EDTA-γδ(141—183) (2.0 μM); and *lanes 7* and *8*, EDTA-γδ(141–183) (10.0 μM). *Lanes 9* and *10* are MPE·Fe footprinting lanes, γδ(141–183) 2.0 μM. *Lanes 11* and *12* are MPE·Fe control lanes. *Lanes 13* and *16* are 5′ and 3′ chemical sequencing G reactions, respectively (Maxam and Gilbert, 1980); *lanes 14* and *15* contain 5′ and 3′ chemical sequencing A reactions, respectively (Iverson and Dervan, 1987).

groove in which the EDTA·Fe is located can be identified by analysis of the cleavage patterns. An EDTA·Fe located in the minor groove generates an asymmetric cleavage pattern with maximal cleavage loci shifted to the 3′ side on opposite strands (Fig. 1). When the EDTA·Fe is located in the major groove, the maximal cleavage loci are 5′-shifted; in addition, cleavage of lower efficiency occurs on the distal strands of the adjacent minor grooves. This results in a pair of 3′-shifted asymmetric cleavage loci of unequal intensity on opposite strands (Fig. 1). These patterns can be explained if the diffusible radical generated from the localized EDTA·Fe reacts in the major and minor grooves of DNA with unequal rates, preferentially (although not necessarily exclusively) in the minor groove.

We report here chemical syntheses of the 43-residue DNA binding domain of γδ with EDTA at the NH_2 terminus, the COOH terminus, and both termini. Affinity cleaving studies using Fe·EDTA-γδ(141–183) reveal that the NH_2 terminus of the DNA binding domain of γδ lies proximal to the minor groove near the center of γδ recombination sites. Attachment of EDTA near the COOH terminus of γδ(141–183) reveals that the recognition helix is oriented toward the center of the inverted repeats, in a manner similar to that seen in the 434 and λ repressor-DNA co-crystals. The location of the cleavage patterns reveal a structural motif very similar to the DNA binding domain of *Hin* recombinase, *Hin*(139–184) (Sluka *et al.*, 1987, 1990a; Mack *et al.*, 1990).

EXPERIMENTAL PROCEDURES

Materials—Manual peptide syntheses were carried out in 20-ml vessels fitted with a coarse glass frit as described by Kent (1988). Automated syntheses were performed on an ABI 430A synthesizer (Kent *et al.*, 1984, 1985), modified by the removal of in-line filters to the top and bottom of the reaction vessel, using a 20-ml Teflon/KelF reaction vessel. The synthetic protocols used were developed at the California Institute of Technology (Kent and Clark-Lewis, 1985; Clark-Lewis *et al.*, 1986; Kent *et al.*, 1988; Kent, 1988). Protected amino acid derivatives were purchased from Peninsula Laboratories. Boc-L-His (DNP) was obtained from Fluka and *N*-α-*t*-Boc-*N*-ε-Fmoc-L-lysine from Chemical Dynamics Corp. (S. Plainfield, NJ). Phenylacetamidomethyl resin with *N*-α-*t*-Boc-L-asparagine was purchased from Applied Biosystems (Foster City, CA) and *p*-methylbenzhydrylamine resin was purchased from United States Biochemical Corp. Dimethylformamide (DMF), diisopropylethylamine (DIEA), dicyclohexylcarbodiimide in dichloromethane, *N*-hydroxybenzotriazole (HOBt) in DMF, and trifluoroacetic acid were obtained from Applied Biosystems. Dichloromethane (DCM) and methanol (HPLC grade) were purchased from Mallinckrodt, 1,4-butanedithiol and anisole from Aldrich, and diethyl ether (low peroxide content) from J. T. Baker Chemical Co. Doubly distilled water was used for all aqueous reactions and dilutions. Calf thymus DNA was purchased from Sigma and sonicated, deproteinized, and dialyzed. Enzymes were purchased from Boehringer Mannheim or New England Biolabs.

Synthesis—*N*-α-Boc-L-amino acids were used with the following side chain protecting groups, Arg(Tos), Asp(OBzl), Glu(OBzl), His(DNP), Lys(Cl-Z), Ser(Bzl), Trp(formyl), Thr(Bzl), and Tyr(Br·Z). Manual assembly of the protected peptide on the solid support was carried out as described previously (Sluka *et al.*, 1990a; Mack *et al.*, 1990). Automated syntheses were carried out with modified cycles which are similar to the manual procedures (Kent, 1988). Double couplings were performed for every amino acid. Boc protecting groups were removed from the α-amino group of the resin-bound amino acid using 100% trifluoroacetic acid. The deprotected peptide resin was neutralized with 10% DIEA in DMF. Amino acids (except asparagine, glutamine, and arginine) were coupled to the free α-amino group as the symmetric anhydrides. In the first coupling the symmetric anhydride was formed in an activating vessel with dicyclohexylcarbodiimide in DCM. The dicyclohexylurea was removed by filtering the solution into a concentrating vessel, where the DCM was removed and replaced with DMF. The solution was then transferred to the reaction vessel where the resin previously had been deprotected and neutralized. After coupling, the resin was neutralized with DIEA in DMF for the second coupling. The symmetric anhydride was formed in the activator in DCM, filtered, and transferred to the reaction vessel. DMF was added at the midpoint of the reaction cycle. Yields for double couplings were determined by quantitative ninhydrin monitoring (Sarin *et al.*, 1981).

Asparagine, glutamine, and arginine were coupled as the HOBt esters in DMF. For the first coupling the dicyclohexylcarbodiimide in DCM was transferred to the concentrator, and the solvent was exchanged for DMF. HOBt and the amino acid in DMF were then added to the concentrator, and the active ester was allowed to form. The ester was transferred to the reaction vessel where the amino acid was allowed to couple. After coupling the resin was neutralized with DIEA in DMF for the second coupling. The second coupling was identical to the first except that the DCM was not removed from the activating solution until after the ester formed. The second coupling for arginine was identical to the first symmetric anhydride coupling. Reaction times for HOBt esters were longer than the symmetric anhydride due to the slower coupling reaction. In a single synthesis, a resin-bound peptide corresponding to the residues 141–183 of γδ-resolvase was produced. Coupling efficiences ranged from a high of 99.8% near the beginning of the synthesis to a low of 98.0% at the end. For γδ(141–183) the average yield for 42 couplings was 99.3%.

EDTA was attached to the NH_2 terminus of the protected peptide as the tribenzylester with a γ-aminobutyric acid linker via an HOBt

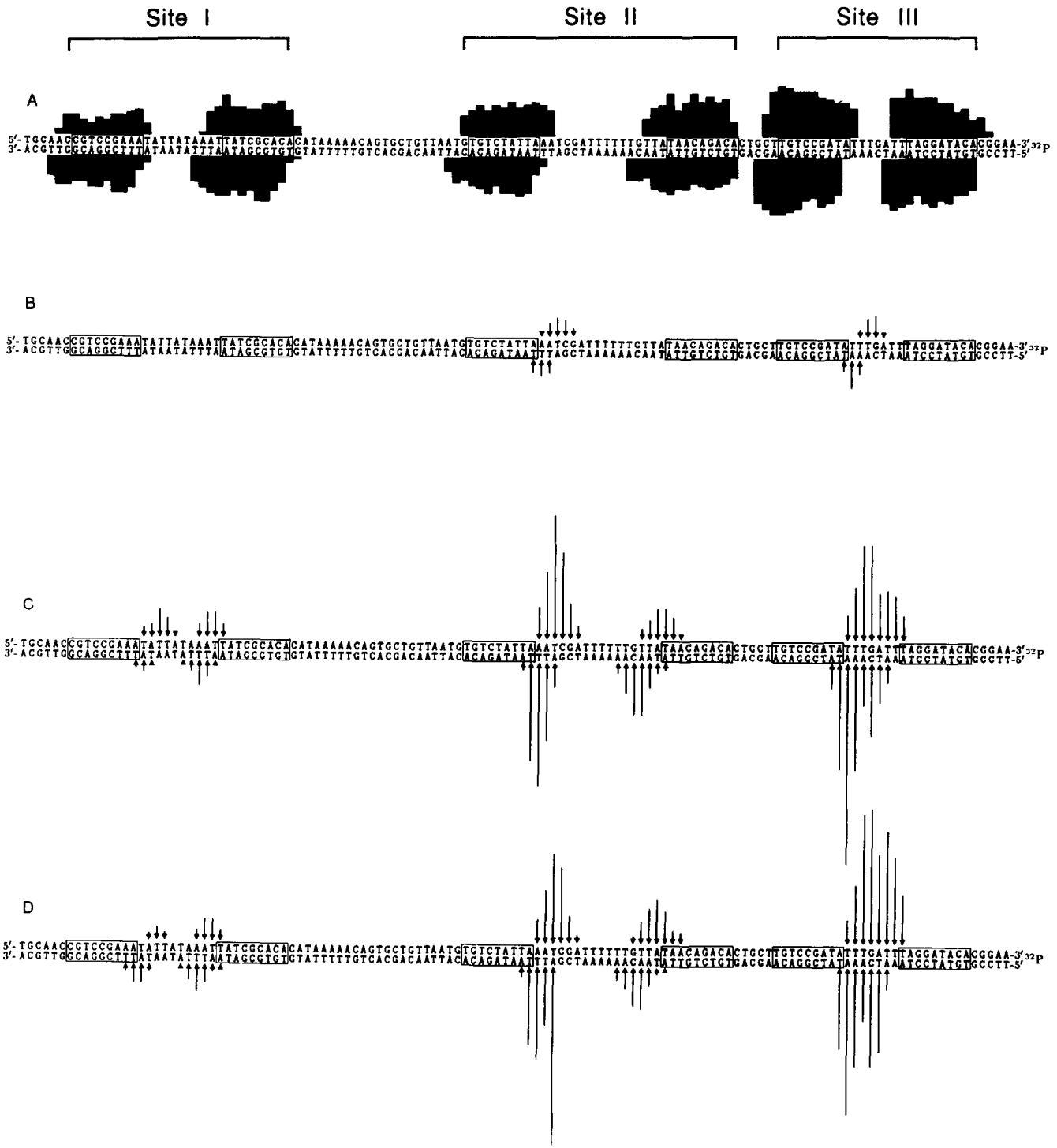

FIG. 5. **Histograms of footprinting and affinity cleaving data from Fig. 4.** The sequence left to right corresponds to the DNA sequence for Site I to Site III (*top* to *bottom* of the gel). Sites I, II, and III are indicated by *brackets*. *Boxes* represent the binding sites assigned for γδ-resolvase (Grindley *et al.*, 1982). *A*, *bars* represent the extent of protection from MPE·Fe cleavage in the presence of γδ(141–183) (2.0 μM). *B*, *arrows* represent the extent of cleavage for Fe·EDTA-γδ(141–183) at 0.5 μM. *C*, 2.0 μM; *D*, 10.0 μM.

ester (Sluka *et al.*, 1987, 1990b). EDTA was positioned at the COOH terminus by covalent attachment to a lysine side chain (Asn^{183}→Lys^{183}) (Mack *et al.*, 1990; Sluka *et al.*, 1990b). *N*-α-*t*-Boc-*N*-ε-Fmoc-L-lysine was activated with dicylcohexylcarbodiimide and coupled onto the *p*-methylbenzhydrylamine resin. Selective removal of the Fmoc protecting group was accomplished with 20% piperidine in DMF for 20 min (Stewart and Young, 1981). The tricyclohexylester of EDTA (Sluka *et al.*, 1990b) was then coupled to this amine as the HOBt ester (Sluka *et al.*, 1990b). γδ(141–183, Asn^{183}→Lys^{183}) equipped with EDTA at both termini was synthesized by the combination of the procedures described above.

Protein Deprotection and Purification—The histidine protecting group, dinitrophenol (DNP), was removed at 25 °C using 20% 2-mercaptoethanol and 10% DIEA in DMF; this treatment was repeated twice (two times for 30 min). After removal of the *N*-α-*t*-Boc group with trifluoroacetic acid and drying of the resin, all other side chain protecting groups were removed, and the peptide-resin bond was cleaved using anhydrous HF in the presence of anisole and 1,4-butanedithiol as scavengers for 60 min at 0 °C. The HF was removed under vacuum. The crude protein was precipitated with diethyl ether,

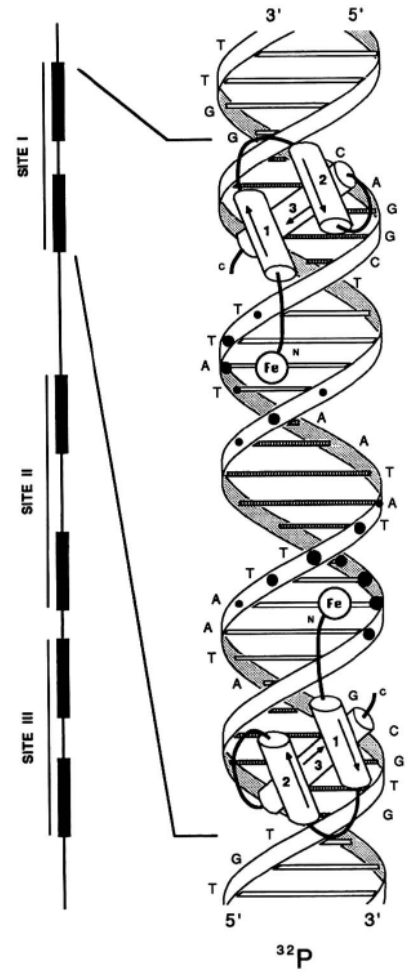

FIG. 6. **Model for γδ(141–183) binding to Site I of *res*.** The location of the EDTA·Fe moiety at the NH_2 terminus of γδ(141–183) was assigned from the cleavage patterns for Fe·EDTA-γδ(141–183). *Filled circles* represent the positions of cleavage along the phosphodiester backbone. Sizes of circles represent the extent of cleavage at the indicated base position.

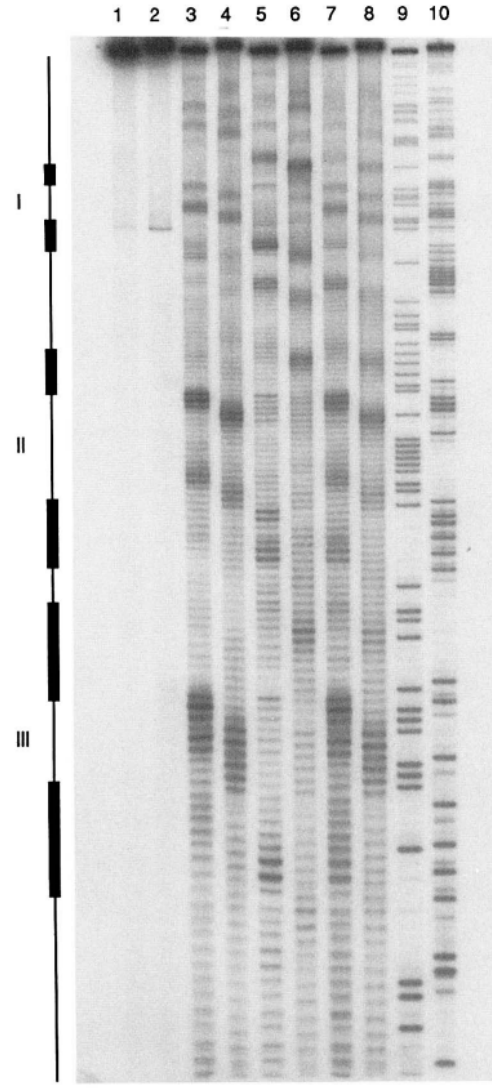

FIG. 7. **Autoradiogram of a high resolution denaturing polyacrylamide gel.** Affinity cleaving reactions by γδ(141–183)-EDTA·Fe on a ^{32}P-end-labeled fragment containing *res*. *Odd-numbered lanes 1–9* contain 5′-labeled DNA and *even-numbered lanes 2–10* contain 3′-labeled DNA. *Lanes 1* and *2*, intact DNA, *lanes 3* and *4*, 10 μM Fe·EDTA-γδ(141–183); *lanes 5* and *6*, 10.0 μM γδ(141–183)-EDTA·Fe; *lanes 7* and *8*, 10.0 μM Fe·EDTA-γδ(141–183)-EDTA·Fe; *lanes 9* and *10*, specific marker lanes (Iverson and Dervan, 1987). *Bars on left* mark the location of half-sites for sequence specific binding.

collected on a fritted funnel, dissolved with 5% acetic acid, and washed through, leaving the resin on the frit. A small sample was then removed, filtered, and subjected to analytical HPLC (Brownlee 25 cm × 4.6 mm C_8 column, 0–60% acetonitrile, 0.1% trifluoroacetic acid over 60 min). The remaining solution was frozen and lyophilized. Residual DNP groups were removed from the crude peptide by treatment in 4 M guanidine HCl, 50 mM Tris, pH 8.5, and 20% 2-mercaptoethanol for 1 h at 50 °C (Kent, 1988). This solution was injected directly onto a semipreparative C_8 HPLC column (25 × 1 cm) and run in H_2O, 0.1% trifluoroacetic acid until the guanidine and 2-mercaptoethanol had eluted. A gradient of 0–60% acetonitrile, 0.1% trifluoroacetic acid was run over 240 min, and fractions were collected. Fractions were analyzed by HPLC. Sequencing by Edman degradation and amino acid analysis of the purified peptide showed its composition to be identical to the sequence for γδ(141–183). Protein concentrations were assayed based on calculated OD_{275} (ε = 6950 based on one tyrosine and one tryptophan). The purified proteins were lyophilized for storage.

DNA Cleavage Reactions—The plasmid PRW80 (Abdel-Meguid *et al.*, 1984) containing two copies of *res* was digested with restriction endonuclease *Hin*dIII to afford two fragments, 4 and 3 kilobase pairs in size. Labeling at the 3′ end was accomplished with [α-^{32}P]dATP using the Klenow fragment of DNA polymerase I. The 5′ end was labeled with ^{32}P by dephosphorylation with calf alkaline phosphatase followed by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase. Cleavage with restriction endonuclease *Sal*I yielded three different size 3′- and 5′-labeled fragments. The smallest fragment, 240 bp long, containing the intact *res* site was isolated by nondenaturing polyacrylamide gel electrophoresis.

MPE footprinting reactions were performed in a buffer containing 20 mM NaCl, 100 μM in base pair calf thymus DNA, 20 mM Tris, pH 8.0, and ^{32}P-end-labeled DNA. The reactions contained MPE·Fe(II) (10 μM), DTT (5 mM), and γδ(141–183) (2.0 μM). For footprinting reactions, γδ(141–183) was added to the buffer and allowed to equilibrate with the DNA for 10 min. MPE·Fe(II) was added and allowed to equilibrate for 5 min. The reaction was initiated by the addition of DTT. After 10 min at 25 °C the reaction was stopped by ethanol precipitation.

DNA cleavage reactions were run in a total volume of 10 μl. Final concentrations were 20 mM Tris, pH 8.0, 20 mM NaCl, 100 μM in base pairs calf thymus DNA, ≈15,000 cpm of ^{32}P-end-labeled restriction fragment, 1 mM dithiothreitol, and 0.5, 2.0, and 10 μM Fe·EDTA-protein. The proteins were allowed to equilibrate with the DNA for 10 min at 25 °C, cleavage was then initiated by the addition of DTT and allowed to proceed for 45 min at 25 °C. The ^{32}P-labeled DNA products were analyzed by denaturing polyacrylamide gel electrophoresis followed by autoradiography. Densitometric analysis of the gel autoradiogram and comparison of individual lanes with sequence marker lanes (Maxam and Gilbert, 1980; Iverson and Dervan, 1987) allowed assignment of DNA cleavage to nucleotide resolution.

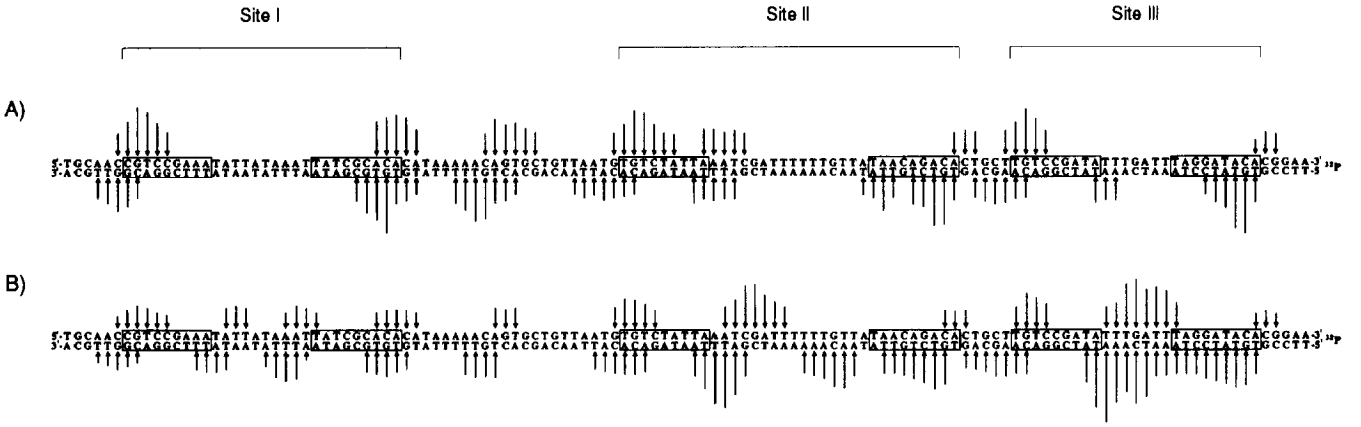

FIG. 8. **Histogram of the affinity cleavage data from Fig. 7.** The sequence left to right represents sites I, II, and III (*top* to *bottom* of the gel). *Arrow* heights indicate the extent of cleavage at the indicated bases. *A*, Cleavage by γδ(141–183)-EDTA·Fe. *B*, cleavage by Fe·EDTA-γδ(141—183)-EDTA·Fe.

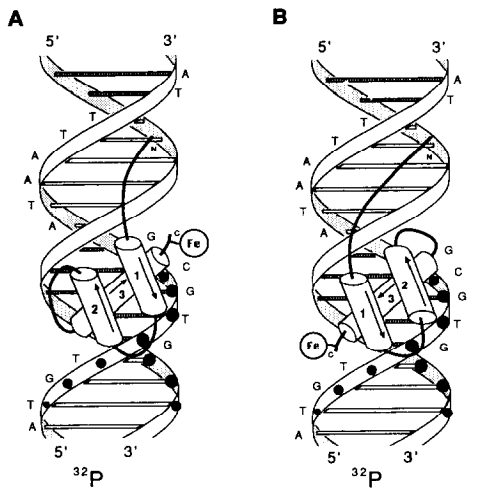

FIG. 9. **Schematic representation of two models of γδ(141–183)-EDTA·Fe bound to the right-half of site I with *circles* indicating sites of maximal cleavage produced by γδ(141–183)-EDTA•Fe.** Sizes of circles represent the extent of cleavage along the phosphodiester backbone. *A* represents the orientation of the putative recognition helix based on 434- and λ-repressor-DNA cocrystals. *B* represents an orientation based on *lac* repressor. The model on the *left* (*A*) seems to fit the experimental cleavage data best.

RESULTS AND DISCUSSION

Synthesis—Four 43-residue proteins, based on the DNA binding domain of γδ-resolvase (residues 141–183), were synthesized by automated solid-phase techniques using *t*-Boc-protected amino acids: one with EDTA at the NH_2 terminus, EDTA-γδ(141–183); one with EDTA at the COOH terminus (Asn^{183}→Lys^{183}), γδ(141–183)-EDTA; one with EDTA at both the NH_2 and COOH termini, EDTA-γδ(141–183)-EDTA; and one with no modification at either termini (Fig. 2). Tribenzyl EDTA-γ-aminobutyric acid was attached to the NH_2 terminus of the protected peptide-resin as described (Sluka *et al.*, 1987, 1990b) to afford EDTA-γδ(141–183). Attachment of EDTA near the COOH terminus of the protein was accomplished by a combination of *t*-Boc and Fmoc protection schemes (Fig. 3) (Mack *et al.*, 1990; Sluka *et al.*, 1990b). *N*-ε-Fmoc-*N*-α-*t*-Boc lysine was substituted for Asn^{183} of γδ(141–183). The Fmoc-protecting group was then removed selectively from the ε-NH_2 chain using piperdine in DMF. Attachment of the tricyclohexyl ester of EDTA to Lys^{183} and completion of the synthesis afforded γδ(141–183)-EDTA.

Footprinting—Footprinting studies of the synthetic protein, γδ(141–183), demonstrated that at 2.0 μM concentrations the 43-mer binds to all six half-sites contained within the *res* binding site (Figs. 4 and 5). At each half-site the synthetic γδ(141–183) protects a 12-bp region of DNA centered on the consensus binding sequence (Figs. 4 and 5). These observations are consistent with DNase I footprinting studies (Abdel-Meguid *et al.*, 1984) and ethylation interference studies (Rimphanitchayakit *et al.*, 1989) using γδ(141–183) derived from a chymotrypsin digest of native γδ-resolvase.

Position of the NH_2 Terminus—Affinity cleaving studies with Fe·EDTA-γδ(141–183) yields a 3′-shifted cleavage pattern at each half-site located at the center of each binding site (Fig. 5). The 3′ shift of the cleavage pattern indicates that the Fe·EDTA group at the NH_2 terminus of γδ(141–183) is located proximal to the minor groove of DNA near the center of the dimeric binding sites (Fig. 6). This is in agreement with the ethylation interference studies (Rimphanitchayakit *et al.*, 1989). The cleavage of *res* by Fe·EDTA-γδ(141–183) at different concentrations (0.5–10 μM) shows that the six half-sites have different affinities for the DNA binding domain. At 0.5 μM concentration, sites II-L and site III-L are cleaved. At 4-fold higher concentrations (2.0 μM), cleavage at sites I-L and I-R, II-R, III-R appear with unequal intensity (Fig. 5). If the amount of cleavage is proportional to the extent of site occupancy, the data suggest that the relative affinities of the DNA binding domain of γδ is II-L, III-L > III-R, II-R > I-L,I-R. This is somewhat different than that assigned from footprinting studies of the DNA binding domain obtained by chymotrypsin digest, II-L, III-L > I-R > III-R,I-L > II-R. (Abdel-Meguid *et al.*, 1984).

Position of the COOH Terminus—The specific cleavage patterns produced by γδ(141–183)-EDTA·Fe are shifted to the 3′ side and indicates that the EDTA·Fe attached near the COOH terminus of the putative recognition helix is positioned within the γδ-binding site above the minor groove of sequence 5′-TGTGC-3′ (Fig. 7, *lanes 5* and *6*; Fig. 8*A*). Two possible orientations for the putative recognition helix of γδ(141–183) can be considered, one oriented toward and one away from the center of the binding site (Fig. 9, *A* and *B*). When the position of the EDTA·Fe-Lys^{183} is considered in each case relative to the cleavage data, a better fit is obtained with orientation 9*A*. This is similar to the orientation of the recognition helix of the λ- and 434-repressors (Aggarwal *et al.*, 1988; Jordan and Pabo, 1988) and that assigned for the 52-residue DNA binding domain of *Hin* recombinase (Mack *et al.*, 1990). The fact that the cleavage pattern is seen predominately *on one but not both* minor grooves adjacent to the

major groove location of the helix-turn-helix motif suggests that the EDTA·Fe moiety (and, hence the putative "recognition helix") is not positioned symmetrically in the major groove. The data would be consistent with the COOH end of the "recognition helix" projecting outward from the floor of the major groove and tilting away from the center of the inverted repeat binding site. Clearly, refinement of these models must await more definitive x-ray crystallographic and nuclear magnetic resonance analyses of the protein·DNA complex.

In controls, γδ(141–183) equipped with EDTA at both the NH_2 and COOH termini, Fe·EDTA-γδ(141–183)-EDTA·Fe affords a pair of cleavage patterns consistent with the combination of patterns from Fe·EDTA-γδ(141–183) and γδ(141–183)-EDTA·Fe (Fig. 7, *lanes 7* and *8*; Fig. 8*B*). The fact that the cleavage pattern for EDTA·Fe at Gly^{141} (NH_2 terminus) appears unchanged when EDTA·Fe is present or absent at the COOH terminus suggests that γδ(141–183) maintains the same structure independent of which termini is modified with EDTA·Fe.

Conclusion—MPE·Fe(II) footprinting studies have shown that the protein protects a 12-bp region of DNA centered on the γδ recognition sequence. Affinity cleaving studies with EDTA on the NH_2 terminus have located the NH_2 terminus of γδ(141–183) in the minor groove at the center of each binding site. Cleaving studies with EDTA attached at the COOH terminus of γδ(141–183) reveal that the putative recognition helix is in the adjacent major groove, oriented (N→C) toward the center of each binding site. A binding model for γδ(141–183), similar to that proposed for *Hin*(139–190) (Sluka *et al.*, 1987, 1990a; Mack *et al.*, 1990), includes a helix-turn-helix motif in the major groove with residues at the NH_2 terminus extending across the DNA phosphodiester backbone and making specific contacts on the same face of the helix to the adjacent minor groove. γδ and *Hin* DNA binding domains may be examples of modular DNA and protein interactions with two adjacent DNA sites (major and minor grooves) bound on the same face of the helix by two separate parts of the protein.

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